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Taxonomy and chemical aspects of *Psilocybe wrightii* from southern Brazil

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Abstract — *Psilocybe wrightii* (*Strophariaceae, Agaricales*), a subtropical and hallucinogenic species known only from southern Brazil and northern Argentina, is confirmed to contain psilocybin and psilocin. Detailed description of southern Brazilian specimens, chemical analysis using gas chromatography of the basidiomes, and discussion of its taxonomy are presented.

Key words — *Psilocybe* sect. *Cordisporae,* screening, toxic mushroom

Introduction

Species of *Psilocybe* (Fr.) P. Kumm. are the most important hallucinogenic mushrooms in the world (Guzmán 1983, Stamets 1996), comprising about 150 neurotropic species, among 227 taxa worldwide (Guzmán 2005). Most of the so-called hallucinogenic species are included in *Psilocybe* sections *Aztecorum* Guzmán, *Brunneocystidiatae* Guzmán, *Cordisporae* Guzmán, *Cubensis* Guzmán, *Mexicanae* Guzmán, *Semilanceatae* Guzmán, *Stuntzii* Guzmán, and *Zapotecorum* Guzmán, following infrageneric classification of Guzmán (1983, 1995). However, many of these species have not been chemically studied, although they are considered to be neurotropic due to the bluing feature of the basidiomes when injured or touched (Guzmán 1983). The bluing color is the oxidative reaction of the main toxins involved —psilocybin and psilocin (Benedict & Tyler 1967). Molecular systematics research supports separation of psilocybinic taxa of *Psilocybe* (clade '/psychedelia' of Moncalvo et al. 2002) from the non-bluing species (Matheny et al. 2006). Redhead et al. (2007) propose conserving the genus *Psilocybe* with *P. semilanceata* (a hallucinogenic

species) as conserved type; the name *Deconica* (W.G. Sm.) P. Karst. would then be available for the non-hallucinogenic clade.

Guzmán (1978) was the first to study the Brazilian *Psilocybe* mycobiota. Present knowledge is summarized by Guzmán & Cortez (2004), who cite 29 species, including 21 neurotropic taxa. In the same paper, the authors report *P. wrightii*, a species known until then only from the holotype, collected in 1971 in the region of Tucumán, Argentina (Guzmán 1978). This mushroom was found to be common near small rivers and very humid places in southern Brazil, where medium-sized basidiomes exhibited the features typical of the bluing species (Silva et al. 2006). This investigation was proposed to provide further taxonomic and chemical data on this species, especially regarding the presence of hallucinogenic compounds.

Materials and methods

Samples – Specimens were collected in the municipality of Santa Maria, central Rio Grande do Sul State, in southern Brazil. The specimens were photographed in situ, collected, and then analyzed macro- and micromorphologically following previously described methods (Guzmán 1983). Color codes follow Kornerup & Wanscher (1978). A minimum of 25 microstructures (basidia, basidiospores, cystidia and hyphae) were observed, which were drawn with the aid of a light tube at magnification of 1000x. The studied collections are preserved at the herbarium ICN (Instituto de Biociências, Universidade Federal do Rio Grande do Sul).

Chemical analysis – The hallucinogenic compounds (e.g., psilocybin and psilocin) were extracted through decoction using previously dried mushrooms (100 mg) and methanol (10 mL), 70°C for 30 minutes so that the resulting extract could be analysed without preconcentration. Psilocybin and psilocin detection methods include thin layer chromatography employing silica gel GF_{254} and methanol : NH_4OH (10:0.25) as mobile phase and platinized potassium iodine as the chromogenic reagent. These samples were analyzed by gas-chromatography-mass spectrometry (GC/MS) on a chromatograph (Shimadzu GC-17A) equipped with a fused silica capillary column 30 m × 0.25 mm × 0.25 µm, coated with DB-5 and a quadrupole MS system (QP 5000) operating at 70 eV. Injector temperature was set at 250 °C. The oven temperature was programmed from 180°C to 320°C at 20°C/min and helium was employed as carrier gas (1 mL/min). Percentage compositions were obtained from electronic integration measurements without taking into account relative response factors. Prior to GC analyses, the methanolic extracts were submitted to derivatization with BSTFA (N-O-bis-trimethylsilyl-trifluoracetamide) following methods adapted from Keller et al. (1999).

Fig. 1. Fresh basidiomes of *Psilocybe wrightii*.

Results and discussion

Psilocybe wrightii Guzmán, Mycotaxon 7: 251, 1978. Fig. 1–6

Pileus 28–49 mm diam., convex to applanate, umbonate, golden brown (5D8) to yellowish brown (5C7) or light orange (5A4) toward the margin, which can present greenish tones; surface smooth, dry to moist, and strongly hygrophanous; margin smooth to striate when dried or striatulate and translucent when moist, appendiculate; context whitish, becoming blue after a few minutes (not readily staining); odor strongly farinaceous. Lamellae adnexed, greyish yellow (4C6) when young, violet brown (11F8) when mature, with conspicuously whitish margin, close. Stipe 52–74 × 3–6 mm, central, cylindrical to subclavate, light orange (5A5) to reddish brown (8D6), base white, greenish tones seen in older basidiomes or within a few minutes after injured; surface smooth in the apex becoming squamulose toward the bottom, hollow. Veil not forming an annulus on stipe but producing marginal membranous remnants on pileus, first yellowish then bluish to greenish in older specimens. Spore print dark purple (14F5).

Basidiospores 7.5–9 × 5.5–6.5 × 5–6 µm, subrhomboid in face view, ovoid to subellipsoid in side view, yellowish brown in KOH, smooth and thickened walls (<1 µm thickness), with a small but truncate germ pore. Basidia 17–23.5 × 6–8.5 µm, ventricose to subclavate, with central constriction, tetrasporic, hyaline. Pleurocystidia 12.5–20.5 × 6–9 µm, fusoid to ventricose, apex mucronate or subcapitate, hyaline, but with the apex slightly pigmented and sometimes chrysocystidioid due to the presence of small and yellow contents, walls thin. Cheilocystidia (13.5–)19–23.5(–28) × 3.5–7.5 µm, lageniform to ventricose, apex sometimes forked, hyaline, with pigmented apex, very numerous making the gill edge completely sterile. Caulocystidia 20–29.5 × 5–7 µm, mainly clavate, sometimes forked, apex obtuse, occasionally mucronate, hyaline, in small clusters on stipe apex. Pileipellis an ixocutis formed by filamentous, little gelatinized, 2–4 µm diam. hyphae, hyaline and with smooth and thin walls. Context subcellular, formed by subglobose, 8.5–31 µm diam. elements, with yellow and slightly thickened walls. Lamella trama subregular, composed by hyaline to pale yellow hyphae, 5–15 µm diam., with smooth and thin walls. Stipitipellis composed by hyaline hyphae, 6–11 µm diam., smooth and thin-walled. Clamp connections present in most septa.

Specimen examined: **BRAZIL**. Rio Grande do Sul: Santa Maria, Três Barras, 14 Mar 2008, *V.G. Cortez 064/08* (ICN).

Taxonomic discussion – *Psilocybe wrightii* was described from Argentina (Guzmán 1978) and later reported from southern Brazil (Guzmán & Cortez 2004), where it has been recently encountered in several localities (Silva et al. 2006, 2008). The basidiomes grow solitary or gregarious in riparian forests or

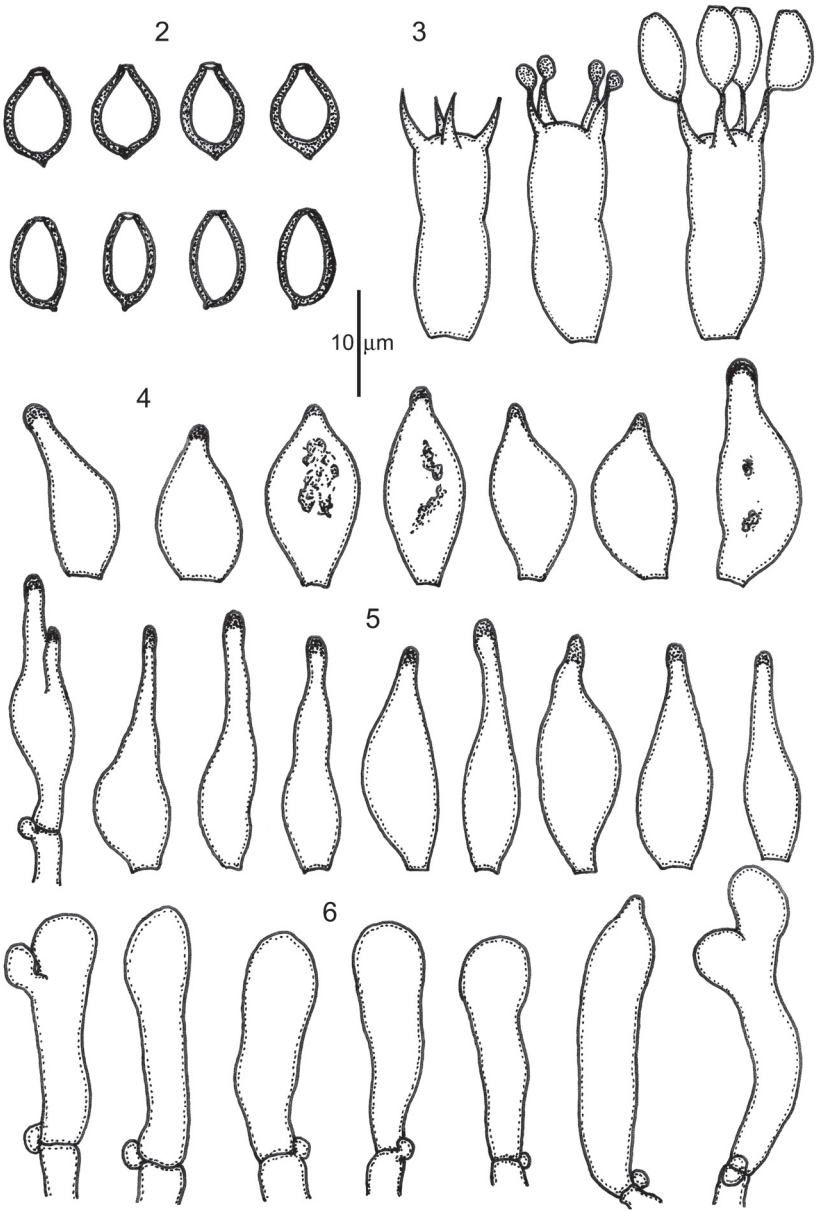

Fig. 2–6. *Psilocybe wrightii*.
2. Basidiospores. 3. Basidia. 4. Pleurocystidia. 5. Cheilocystidia. 6. Caulocystidia.

near small rivers in the autumn (April to June). Based on basidiospore size and shape, presence of pleurocystidia, and the bluing feature of the basidiomes, Guzmán (1983) placed the species in *Psilocybe* Sect. *Cordisporae*, which is widely represented in the Neotropical area.

Psilocybe heliconiae Guzmán et al., described from Colombia, shares similar size and shape of the basidiospores, pleurocystidia and cheilocystidia as well as similarities in the basidiome appearance (Guzmán et al. 1994). This similarity was emphasized in Guzmán et al. (1994) and Guzmán & Cortez (2004), but only with evaluation of additional collections to establish the variation from young to mature basidiomes can the taxonomic relationships between these species be clarified.

Chemical analysis – After extraction, on sorting analysis, all the samples showed an intense characteristic reaction with the chromogenic agent in thin layer chromatography, presenting Rf values of 0.39 for psilocin and 0.5 for psilocybin. Psilocybin and psilocin were also detected through gas chromatography. Derivatization increased the sensitivity of the detector (Lanças 1993). This reaction was performed with BSTFA, producing bis-trimethyl-silyl-psilocybin and bis-trimethyl-silyl-psilocin, which showed a retention time of 10.683 min and 10.592 min, respectively. The identity of these derivatives was confirmed by the presence of the characteristic fragments *m/z* 58, 189 and 261 for bis-trimethyl-silyl-psilocybin and 58, 290 and 348 for bis-trimethyl-silyl-psilocin. Differences in the psilocin and psilocybin contents in hallucinogenic mushrooms depends on factors such as developmental stage, weather conditions, and the availability of soluble nitrogen and phosphorous in the soil (Tsujikawa et al. 2003).

These results confirm the presence of psilocin and psilocybin for the species and, consequently, the hallucinogenic potential of *Psilocybe wrightii*.

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Literature cited

Benedict RG, Tyler VE. 1967. Blueing in *Conocybe, Psilocybe,* and a *Stropharia* species and the detection of psilocybin. Lloydia 30: 149–157.

Guzmán G. 1978. The species of *Psilocybe* known from Central and South America. Mycotaxon 7: 225–255.

Guzmán G. 1983. The genus *Psilocybe.* Beih. Nova Hedw. 74: 1–439.

Guzmán G. 1995. Supplement to the monograph of the genus *Psilocybe.* Bibl. Mycol. 159: 91–141.

Guzmán G. 2005. Species diversity of the genus *Psilocybe* (*Basidiomycotina, Agaricales, Strophariaceae*) in the world mycobiota with special attention to hallucinogenic properties. Int. J. Med. Mushr. 7: 305–331.

Guzmán G, Cortez VG. 2004. The neurotropic *Psilocybe* (Fr.) P. Kumm. (*Strophariaceae, Agaricales*) in Brazil: a revision of the known species, the first record of *P. wrightii* and the synonymy of *P. caeruleoannulata*. Int. J. Med. Mushr. 6: 383–388.

Guzmán G, Saldarriaga Y, Pineda F, García G, Velázquez LF. 1994. New species of *Psilocybe* from Colombia and discussion on the known species. Mycotaxon 51: 225–235.

Keller T, Schneider A, Regenscheit P, Dirnhofer R, Rücker T, Jaspers J, Kisser W. 1999. Analysis of psilocybin and psilocin in *Psilocybe subcubensis* Guzmán by ion mobility spectrometry and gas chromatography-mass spectrometry. Forensic Sci. Int. 99: 93–105.

Kornerup A, Wanscher JH. 1978. Methuen handbook of colour. 3rd ed. London (UK), Eyre Methuen.

Lanças FM. 1993. Cromatografia em fase gasosa. São Carlos (Brazil), Acta.

Matheny PB, Curtis JM, Hofstetter V, Aime MC, Moncalvo JM, Ge ZW, Yang ZL, Slot JC, Ammirati JF, Baroni TJ, Bougher NL, Hughes KW, Lodge DJ, Kerrigan RW, Seidl MT, Aanen DK, DeNitis M, Daniele GM, Desjardin DE, Kropp BR, Norvell LL, Parker A, Vellinga EC, Vilgalys R, Hibbett DS. 2006. Major clades of *Agaricales:* a multilocus phylogenetic review. Mycologia 98: 982–995.

Moncalvo JM, Vilgalys R, Redhead SA, Johnson JE, James TY, Aime MC, Hoffstetter V, Verduin SJW, Larsson E, Baroni TJ, Thorn RG, Jacobsson S, Clémençon H, Miller Jr. OK. 2002. One hundred and seventeen clades of euagarics. Mol. Phylogen. Evol. 23: 357–400.

Redhead SA, Moncalvo JM, Vilgalys R, Matheny PB, Guzmán-Dávalos L, Guzmán G. 2007. Proposal to conserve the name *Psilocybe* (*Basidiomycota*) with a conserved type. Taxon 56: 255–257.

Silva PS, Cortez VG, Silveira RMB. 2006. The mycobiota of Itapuã Park, Rio Grande do Sul, Brazil. I. Species of *Strophariaceae* (*Agaricales*). Mycotaxon 97: 219–229.

Silva PS, Cortez VG, Silveira RMB. 2008. *Strophariaceae* (*Agaricales, Basidiomycota*) no Parque Estadual de Itapuã, Viamão, Rio Grande do Sul: chave para identificação das espécies. Rev. Bras. Bioc. 6: 253–258.

Stamets P. 1996. Psilocybin mushrooms of the world. Berkeley (USA), Ten Speed Press.

Tsujikawa K, Kanamori T, Iwata Y, Ohmae Y, Sugita R, Inoue H, Kishi T. 2003. Morphological and chemical analysis of magic mushrooms in Japan. Forensic Sci. Int. 138: 85–90.